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| (54) Title: AUTOCRINE MOTILITY FACTORS IN CANCER DIAGNOSIS AND MANAGEMENT (57) Abstract The present invention describes an isolated and substantially pure mammalian cell polypeptide which stimulates random locomotion of producer cell and which has a molecular weight greater than 30,000. The unique polypeptide of the present invention is inhibited by pertussis toxin. A kit and method for detecting metastasis in human are also described. | | |

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1 AUTOCRINE MOTILITY FACTORS IN CANCER
2 DIAGNOSIS AND MANAGEMENT

3 BACKGROUND OF THE INVENTION

4 Technical Field

5 The present invention is related generally to the
6 field of cancer diagnosis and management. More
7 particularly, the present invention is related to novel
8 tumor motility factors and their utility in devising new
9 approaches to cancer diagnosis, prevention and therapy.

10 State of the Art

11 Cell motility is necessary for tumor cells to
12 traverse many stages in the complex cascade of invasion
13 and metastases. Such stages include the detachment and
14 subsequent infiltration of cells from the primary tumor
15 into adjacent tissue, the migration of the cells through
16 the vascular wall into the circulation (intravasation),
17 and extravasation of the cells to a secondary site. The
18 movement of cells through biological barriers such as the
19 endothelial basement membrane of the vasculature may
20 occur by means of chemotactic mechanisms. Studies on in
21 vitro chemotaxis of some tumor cells indicate that a
22 variety of compounds such as complement-derived
23 materials, collagen peptides, formyl peptides, and
24 certain connective tissue components can act as

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1 chemoattractants. Todaro, et al. (Proc. Natl. Acad. Sci
2 USA, 77:5258-5262, 1980) reported autocrine growth
3 factors for transformed cells. Other growth factors of
4 various types are also known. However, the existence and
5 role of an autocrine factor controlling chemotactic
6 (directional) and chemokinetic (random) motility of tumor
7 cells has not heretofore been known or described. It may
8 be important to note here that cell motility is an aspect
9 of cell behavior distinct from cell growth and
10 proliferation.

11 SUMMARY OF THE INVENTION

12 It is, therefore, an object of the present invention
13 to identify and provide an autocrine factor controlling
14 motility of tumor cells, such autocrine factor being
15 designated herein as "AMF."

16 It is a further object of the present invention to
17 provide antibodies having specific binding affinity for
18 AMF or AMF receptors.

19 It is a still further object of the present
20 invention to provide a kit for detecting, localizing and
21 predicting metastases and tumor angiogenesis in humans.

22 It is yet another object of the present invention to
23 provide a method of predicting, preventing and/or
24 treating metastatic invasion and cancer proliferation in
25 humans.

26 It is an additional object of the present invention
27 to provide a pharmaceutical composition comprising an
28 effective amount of neutralizing antibodies against AMF
29 to inhibit motility of tumor cells in a pharmaceutically
30 acceptable carrier.

1 Various other objects and advantages of the present
2 invention will become evident from the Detailed
3 Description of the Invention.

5 These and other objects, features and many of the
6 attendant advantages of the invention will be better
7 understood upon a reading of the following detailed
8 description when considered in connection with the
9 accompanying drawings wherein:

Fig. 2 shows (a) Scatchard analysis of ^{125}I -AMF binding to suspended tumor cells; and (b) dose response curve of cell motility to purified AMF.

16 The above and various other objects and advantages
17 of the present invention are achieved by a polypeptide
18 having the following properties: (a) secreted by
19 mammalian cells and stimulates random locomotion of the
20 producer cells; (b) having molecular weight of $> 30,000$;
21 and (c) being inhibited by pertussis toxin. The
22 polypeptide of the present invention is found to have, at
23 least in part or in whole, the following amino acid
24 sequence at its NH_2 terminus (single letter code) or at
25 the NH_2 terminus of an active fragment of the
26 polypeptide:

27 D K E L R F R D C T K S L A E A N K K

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1 Unless defined otherwise, all technical and
2 scientific terms used herein have the same meaning as
3 commonly understood by one of ordinary skill in the art
4 to which this invention belongs. Although any methods
5 and materials similar or equivalent to those described
6 herein can be used in the practice or testing of the
7 present invention, the preferred methods and materials
8 are now described. All publications mentioned hereunder
9 are incorporated herein by reference.

10 MATERIALS AND METHODS

11 Cell Lines

12 Human MDA231 and MDA435 breast carcinoma cells lines
13 were obtained from ATCC and cultured in Dulbecco's
14 modified Eagle's medium (DMEM) supplemented with 10%
15 fetal bovine serum. Both of these estrogen independent
16 cell lines produce metastases in the lungs of a 6
17 week-old NIH nude mice, 6 weeks following injection of
18 5×10^5 cells into the lateral tail vein.

19 Isolation and Purification of the Autocrine Motility 20 Factor

21 MDA231 and MDA435 human breast carcinoma cells are
22 grown in DMEM to 60% confluency in the absence of added
23 protein. The media is lyophilized and the residue
24 dissolved in about 2 ml of distilled H₂O. This solution
25 is applied to a PD-10 (Sephadex G25 medium) column. The
26 first 2.5 ml are discarded and the next 4 ml are
27 collected. The effluent contains AMF separated from low
28 molecular weight material. This collected fraction is
29 made up to 0.02 M phosphate buffered saline, pH 7.4 (PBS)
30 with 10 x PBS and applied to a Sephacryl S-300 column in
31 PBS (source of column). Elution with PBS

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1 yields an active fraction that corresponds to material
2 with a molecular weight of about 54 kDa. This fraction is
3 dialyzed and concentrated 25 fold. The material is made
4 up to 50 mM Tris-acetate, pH 8.0 and applied to a mono Q
5 anion exchange column (source) and eluted with a linear
6 salt gradient (0-1 M NaCl) with the following
7 modification: When the NaCl concentration reaches
8 0.25 M, this concentration is held for 10 min before
9 resuming the gradient. AMF is eluted in the 0.3 M to
10 0.4 M NaCl fraction. The active fraction is dialyzed
11 and concentrated to a small volume (about 0.5 ml) which
12 in turn is made up to 0.02 M phosphate in normal saline,
13 pH 7.4. This is applied to a heparin column in PBS. The
14 column is eluted with a linear gradient of NaCl (0.15 M
15 to 1 M) which elutes AMF between 0.35 M and 0.4 salt
16 gradient. After each purification step, column fractions
17 (dialyzed to remove salt) are assayed for motility
18 stimulating activity by the modified Boyden chamber
19 procedure.

20 Assay Procedure for Cell Motility

21 The assay of motility is accomplished by the use of
22 a modified Boyden (Zigmond, et al, J. Exp. Med.
23 137:387-410, 1973) chamber. This is a device (Figure 1)
24 consisting of 2 wells horizontally separated by a
25 microporous polycarbonate filter with a pore diameter of
26 about 8 μ . The motility stimulus (or chemoattractant) is
27 placed in the lower well to contact the filter. To the
28 upper well is added a suspension of cells (for example
29 A2085 melanoma cells) at a concentration of about
30 10^6 cells/ml. The chamber is then placed in a humidified
31 incubator for about 4 hours at 37 degrees C in an
32 atmosphere of air and about 5% CO₂. During this time,
33 the cells are deposited by gravity on the topside

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1 of the filter. However, some cells (about 5 to 10%)
2 migrate to the underside of the filter in response to the
3 motility stimulant. Expenditure of energy must occur
4 during migration since the average diameter of the cell
5 is greater than the pore size diameter. At the end of
6 the incubation period, the filter is removed and
7 subjected to a fixing and staining procedure. This
8 includes first immersing the filters in a
9 methanol-containing solution for about 2 minutes; then in
10 an eosin solution for about 2 minutes; and then in a
11 hematoxylin solution for about 3 minutes. Thereafter the
12 filters are washed in water and placed on a glass slide
13 with the topside up. The buttons of stained cells on the
14 topside are completely removed with a small piece of dry
15 tissue paper. The stained cells that have migrated
16 through the filter then become apparent. These are
17 counted with the aid of a microscope at a magnification
18 of about 500X. Five different high power fields are
19 visualized with a grid in one ocular, the cells in 5
20 fields are counted and the average is computed. A ratio
21 of ≥ 5 for positive control/negative control is indicative
22 of a positive response of the cells to the motility
23 stimulus.

24 Determination of Random and Directed (Chemotactic)
25 Motility

26 Measurement of random motility is accomplished by
27 exposing the cells to a fixed concentration of stimulus
28 and determining their migration as described above. This
29 includes adding equal increasing concentrations of
30 attractant to both upper and lower wells prior to the
31 assay incubation. The random migration of cells as a
32 function of the levels of attractant is then determined.
33 Directed migration occurs if the cells migrate better in

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1 positive gradients (higher concentrations of attractant
 2 in the lower well compared to the upper well) than in
 3 negative gradients (higher concentrations in upper well
 4 than in lower well). The results of such an assay are
 5 shown in the "checkerboard" tabulation of the results
 6 (Table 1). It can be seen that random motility is quite
 7 significant for the A2058 melanoma cells responding to
 8 the AMF.

TABLE 1
 % Motility Factor in Upper Well

| | | 0 | 15 | 30 | 45 |
|---------------------------------------|----|------|------|------|------|
| % Motility Factor In Lower Well | 0 | 100 | 244 | 512 | 494 |
| | 15 | 494 | 1056 | 825 | 1469 |
| | 30 | 1781 | 1550 | 2144 | 2640 |
| | 45 | 2800 | 2550 | 2262 | 4362 |

'diagonal' shows random migration of cells. Lower triangle shows directed migration of cells in a positive gradient of motility stimulus.

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1 Assay for Cell Pathways Involved in AMF Induced Motility

2 Materials: DMEM supplements with L-glutamine (2 µg),
3 penicillin and streptomycin with or without 10%
4 heat-inactivated fetal calf serum were purchased from
5 commercial sources such as Meloy Laboratories, Inc.
6 (Springfield, VA). Pertussis toxin and cholera toxin
7 were obtained from List Biological Laboratories, Inc.
8 (Cambell, CA). Phorbol 12-myristate 13-acetate (PMA),
9 phorbol 12, 13-didecanoate (PDD), calcium ionophore
10 A23187, diltiazem, nifedipine, verapamil,
11 trifluoperazine, leupeptin, forskolin and 8-Br cAMP were
12 all purchased from Sigma Chemical Company (St. Louis,
13 MO). The 1-oleoyl-2-acetylglycerol was from Molecular
14 Probes (Eugene, OR). The Nucleopore membranes
15 (polyvinyl- pyrrolidone-free) as well as the 48-well
16 chemotaxis chamber were purchased from Neuro Probe, Inc.
17 (Cabin John, MD).

18 Cell Culture: The human melanoma cell line A2058
19 was maintained as described by Todaro et al, supra.

20 Production of Autocrine Motility Factor: In a
21 modification of the previously described technique
22 (Liotta et al, Proc. Natl. Acad. Sci. USA 83:3302-3306,
23 1986), A2058 cells were inoculated for 48 hours in DMEM
24 without any protein supplement. The medium was
25 concentrated using a Centricon ultrafiltration assembly,
26 molecular weight cut off 30,000 daltons.

27 Chemotaxis Assay: The assay used to determine cell
28 motility was a modification of the techniques described
29 by Harvath et al, 1980... Liotta et al, 1986 supra. In
30 accordance with this technique A2058 melanoma cells
31 (approximately 75-90% confluent) were harvested with
32 trypsin-EDTA and allowed to recover at room temperature
33 in DMEM supplemented with 10% fetal calf serum for at
34 least one hour. The cells were then resuspended at

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1 2×10^6 /ml in DMEM with 1 mg/ml bovine serum albumin.
2 The assay was performed in 48-well micro-chemotaxis
3 chamber (Harvath et al, 1980 supra) with 8 μ m Nucleopore
4 membranes coated with type IV collagen. The chambers
5 were incubated at 37 degrees C for 4-5 hours, then
6 developed using Diff Quick stains (American Scientific).
7 The stained membranes were placed onto glass slides with
8 the original cell side up so that the cell pellet could
9 be wiped from the surface. Cells that had migrated
10 through the pores were trapped between glass and membrane
11 and could be easily counted by light microscopy under
12 high power field (500X). Unstimulated random migration
13 was <20% of directed migration.

14 Prior to or during the chemotaxis assay, chemicals
15 could be co-incubated with cells to alter cellular
16 metabolism or stimulate a chemokinetic response. At the
17 start of the assay, chemicals could also be added to the
18 lower chamber to demonstrate chemotactic potential.

19 Production of Murine Antibodies to AMF

20 Purified AMF protein (10 μ g) was emulsified with
21 complete Freund's adjuvant and injected into the foot pad
22 of 3 C3H mice. Two weeks later the mice were boosted
23 with 5 μ g of AMF in PBS injected intravenously in the
24 tail vein in a volume of 0.1 ml. One month later the
25 mice were bled and the serum was tested for its ability
26 to inhibit tumor cell motility. In this assay the mouse
27 sera was preincubated with the AMF in the Boyden chamber
28 migration assay. At a dilution of 1/1000 the mouse sera
29 produced 90% inhibition of tumor cell motility compared
30 to pooled mouse sera control. Purified AMF protein (10
31 μ g) was emulsified in complete Freund's adjuvant and
32 injected into a subcutaneous site on the back of New
33 Zealand white rabbits. Booster injections of 5 μ g were

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1 applied at 6 and 12 weeks. At 3 and 4 months the rabbits
2 were bled and the sera was tested for motility inhibition
3 activity. At a dose of 1/1000 the immune sera abolished
4 motility compared to control preimmune sera. The sera
5 were heat inactivated at 56°C for 30 minutes.

6 Determination of AMF Purity

7 The purity of the isolated AMF was determined by the
8 following criteria:

9 (a) Single 54 kDA band was found on a single and two
10 dimensional polyacrylamide gel electrophoresis
11 performed by standard procedures well known in the
12 art. Protein was identified with silver stain.

13 (b) Protein band cut from the gel retains motility
14 stimulating activity.

15 (c) NH₂ terminus amino acid sequence (1-19) reveals one
16 type of amino acid residue at each cycle; and

17 (d) Murine and rabbit anti-AMF antibodies block the
18 motility stimulating activity of human tumor AMF.

19 Based on the above criteria, the isolated AMF of
20 the present invention was found to be substantially
21 pure. The term "substantially" as used herein means as
22 pure as it is possible to obtain by standard techniques.

23 Amino Acid Sequencing

24 Edman degradation of purified AMF is performed with
25 the Applied Biosystems (Foster City, CA) model 470A
26 gas-phase sequencer using the trifluoroacetic acid
27 chemistry provided by the manufacturer. The
28 phenylthiohydantoin amino acids were identified and
29 quantitated by using the Perking-Elmer series 3B HPLC and
30 ultraviolet detection.

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1 Dose Response and Time Course of Pertussis Toxin and
2 Effect on Motility: Pertussis toxin (PT) was added to
3 A2058 for overnight culture in flasks, for various period
4 of preincubation prior to an assay, or at different times
5 after the start of an assay. PT doses that were tested
6 ranged from about 10 ng/ml to 1.5 µg/ml. Cell viability
7 at any of the tested doses was comparable to the
8 viability in untreated control (>90%). Treated and
9 untreated cells were then tested for their motility
10 response to the A2058 conditioned medium. Cell motility
11 in response to the DMEM alone was included as a negative
12 control for each treatment group of cells.

13 Overnight incubation of the cells with any of the
14 tested PT doses resulted in significant inhibition of
15 cell motility (Table 2). Preincubation for 30 minutes to
16 2 hours at doses of 0.5 - 1.5 µg/ml also resulted in
17 greater than 50% inhibition. When pertussis toxin was
18 added at the start of the assay or later, there was a
19 gradual diminution in the inhibitory effect. By 1-2
20 hours after the start of the assay, PT had minimal effect
21 on the observed motility.

22 The dose response of PT was consistent with
23 previously described inhibitory doses of PT for G_i and G_o
24 proteins. The time course showed much diminished
25 inhibition when PT was added at inadequate doses or for
26 insufficient time to saturate the G protein sites.
27 Hence, the data obtained in the present testing was
28 consistent with the hypothesis that AMF stimulates cell
29 motility through a receptor which requires a G protein to
30 activate the cells.

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TABLE 2
AMF TREATMENT DATA

| <u>A.</u> | <u>Treatment</u> | <u>Motility (% of Controls)</u> |
|-----------|------------------|---------------------------------|
| 1 | P'ase K | 13.2 |
| 2 | DNAase 2 g/ml | 95.1 |
| 3 | RNAase | 104 |
| 4 | PMSF 5 mM | 95.5 |
| 5 | DDT 10 mM | 11.5 |
| 6 | Heating 100C | 5.0 |
| 7 | Heating 56C | 97.2 |
| 8 | pH 4.0 | 20 |
| 9 | pH 7.4 | 100 |
| 10 | pH 11.0 | 100 |

PERTUSSIS TOXIN INHIBITION OF AMF INDUCED MOTILITY

| <u>B.</u> | <u>Time Pertussis Toxin Added (hrs. from start of assay)</u> | <u>Percent Inhibition of AMF Induced Motility</u> |
|-----------------|--|---|
| | -2.0 | 100 |
| | -1.0 | 95 |
| | -0.5 | 100 |
| Start of Assay* | 0 | 62 |
| | +0.5 | 55 |
| | +1.0 | 33 |
| | +2.0 | 0 (no inhibition)*+ |
| | +3.0 | 0 (no inhibition)*+ |

*Time of addition of AMF

*+Pertussin Toxin requires at least 1 hour to penetrate cell membranes and inhibit G proteins by ADP ribosylation.

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1 Cholera Toxin Dose Response and Time Course: Cholera
2 toxin (CT) in contrast to pertussis toxin, is thought to
3 act on the G_s protein that stimulates adenylate cyclase
4 to produce the second messenger, cAMP. Cholera toxin was
5 added to A2058 cells either for overnight incubation in
6 flasks or for variable periods of preincubation prior to
7 the start of the chemotaxis assay. The tested doses of
8 cholera toxin ranged from about 0.1-50 $\mu\text{g/ml}$. At all
9 tested doses, cell viability was comparable to that of
10 untreated cells (>90%). Treated and untreated cells were
11 then tested for chemotactic response to A2058 conditioned
12 medium.

13 Overnight treatment with CT caused a diminished
14 response to the A2058 conditioned medium, though the
15 inhibitory effect was never complete (30-60%
16 inhibition). If the cells were exposed to cholera toxin
17 for just a brief preincubation prior to the start of the
18 chemotaxis assay, the inhibition was minimal (<5%).

19 Effect of Other Agents Involved in the Adenylate cyclase
20 System on Cell Motility: Cholera toxin is thought to act
21 by ADP-ribosylation of the G_s protein in an active
22 configuration that can stimulate adenylate cyclase.
23 Since the effect of cholera toxin on A2058 cell motility
24 was minimal, further tests were conducted to determine
25 whether other agents that act on the cAMP pathway would
26 be inhibitory. Forskolin stimulates adenylate cyclase
27 directly without acting through an intermediary G
28 protein. The cAMP analogue, 8-Br cAMP, is able to enter
29 intact cells. Both chemicals were added to A2058 cells
30 either for overnight incubation in flasks or for a 2 hour
31 preincubation prior to the start of chemotaxis. Both
32 exhibited only a partial inhibition of cell motility that

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1 was essentially identical to that of cholera toxin for
2 comparable periods of time.

3 Since these cells respond in a dose-dependent manner
4 to various concentration of conditioned medium obtained
5 by incubating confluent cells in serum-free medium, it
6 was concluded that the motility factor is derived from
7 the cell. Results obtained with the modified Boyden
8 chamber experiments also demonstrate that the autocrine
9 factor of the present invention has both chemotactic
10 (directional) and chemokinetic (randomly motile)
11 properties. Since the random stimulation was found to be
12 about three-fold greater than the directed motility, it
13 was concluded that the cells respond to gradients of the
14 motility factor as well as to high uniform concentrations
15 of the attractant.

16 When determined by gel filtration and gel
17 electrophoresis, the migration-stimulating material of
18 the present invention is found to have a molecular weight
19 of about 54 kilodaltons. This form may be a precursor of
20 an active factor. It is possible that cellular or serum
21 components could activate or inhibit the action of the
22 motility factor. The motility factor is inactivated by
23 exposure to streptococcal protease, but active
24 chymotrypsin-derived fragments can be produced (data not
25 shown). The activity is destroyed by boiling but is
26 stable upon exposure to 56 degrees C. Additionally, the
27 activity is stable to a pH range from 4 to 11 (data not
28 shown). These properties indicate that the autocrine
29 material (AMF) of the present invention is different from
30 a variety of known growth factors and chemoattractants.
31 It was also found that known growth factors such as
32 PDGF, α TGF, β TGF, EGF, IGF, transferrin, or FGF do not
33 substitute or block the AMF (data not shown). Amino acid
34 analysis indicated a unique sequence of 19 amino terminal

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1 amino acids of AMF. A slightly small form of the
2 active material was also found to have a unique amino
3 terminal sequence. Protein data base searches failed to
4 reveal any other polypeptide with such a sequence.

5 It has also been found that motility induction by
6 AMF is not blocked or substituted by known growth factors
7 or serum factors. At a concentration of 1 nM or less,
8 AMF markedly stimulates the random and directed motility
9 of breast cancer cells but fails to induce motility in
10 leukocytes. The factor also stimulated random pseudopodia
11 production by breast carcinoma cells and melanoma cells.
12 Following trasfection with the activated ras-oncogene,
13 AMF and its receptor are enhanced more than 100 fold in
14 certain cells. Human breast carcinoma cells, but not
15 normal breast epithelium, produce large quantities of
16 AMF. Antibodies recognizing AMF abolish human tumor cell
17 motility in vitro without altering tumor cell viability.

18 The availability of an isolated and purified
19 autocrine, polypeptide, tumor motility factor makes it
20 possible to obtain anti-AMF antibodies having specific
21 binding affinity for said motility factor. Such
22 antibodies can either be polyclonal or monoclonal and are
23 prepared by well known standard techniques routine in the
24 art. Such antibodies can also be labelled with suitable
25 radioisotopes or fluorescent and other markers or ligands
26 and employed for the detection, quantitation and/or
27 localization of the AMF in human tissue or body fluid.
28 Furthermore, radiolabelled AMF together with unlabelled
29 AMF can be utilized in a standard competitive assay to
30 measure AMF receptor level. Such binding assay for
31 determining the receptor level is carried out as follows.

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1 AMF Binding Assay:

2 Purified AMF is iodinated using the standard Bio Rad
3 enzymobead procedure. Increasing amounts of labeled AMF
4 is incubated in a volume of 1 ml with 100,000 A2058
5 melanoma cells, in the presence or absence of 100 fold
6 excess cold competitor. Incubation is conducted at 37°C
7 for 40 minutes and the cell-bound radioactivity is
8 separated by centrifugation. AMF binding exhibits
9 saturation with 80% specific binding and about 30,000
10 receptors per cell. Scatchard analysis according to
11 standard methods shows a linear relationship between the
12 specifically bound/free ratio and the specifically bound
13 AMF, with an estimated k_d in the range of about 0.5 nM.

14 Detection of cancer in humans is also made possible
15 by the present discovery and testing of human body
16 samples for this purpose is now illustrated using urine
17 samples from bladder cancer patients.

18 Urine samples from patients with bladder cancer are
19 collected and processed with centrifugal
20 microconcentrator (AMICON) with an exclusion filter of 10
21 kilodaltons. The processed urines are reconstituted at a
22 10-fold concentration with sterile phosphate buffered
23 saline pH7.5 and stored at -20°C until use. Tumor grade
24 is determined by a pathologist using a scale of one to
25 three with grade one tumors showing the most
26 differentiation and grade three tumors showing the least
27 differentiation. Bladder tumors are staged according to
28 the American Joint Committee TNM classification.

29 Assay of Urine Samples:

30 Although any cell line which responds to AMF can be
31 employed, the preferred cell line is human MDA 435 cells
32 (ATCC). The concentrated urine samples are applied to the
33 microwell migration chamber assay as described herein

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1 supra. Each sample is tested at a series of dilutions
2 with and without the addition of the antibodies directed
3 against human tumor AMF. AMF units are recorded as the
4 proportion of tumor cells stimulated to migrate by the
5 sample which is inhibited by the antibodies. In general,
6 greater than 80% of the stimulated migration is inhibited
7 by an antibody concentration of about 10 µg/ml.

8 As shown in Table 3, control urines with
9 non-neoplastic disorders such as kidney stones failed to
10 contain significant levels of motility factors. All of
11 the bladder transitional cell carcinoma cases exhibited a
12 positive motility response in the urine. The highest
13 levels of motility factor production was found in the
14 urine of patients with high grade tumors or with stage D
15 (metastatic) tumors.

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TABLE 3

| | <u>Urine Sample</u> | <u>AMF units</u> | <u>SE</u> |
|----|---------------------|------------------|-----------|
| 1 | Control ks 75 | 5 | .5 |
| 2 | Control ks 76 | 9 | 2 |
| 3 | Ca <u>in Situ</u> | 32 | 5 |
| 4 | Papillary TCC | 64 | 8 |
| 5 | TCC 77 | 44 | 3 |
| 6 | TCC 69 | 98 | 14 |
| 7 | TCC 73 | 123 | 32 |
| 8 | Recur TCC 79 | 130 | 22 |
| 9 | TCC II 485 | 169 | 14 |
| 10 | TCC II 491 | 105 | 8 |
| 11 | TCC II 554 | 41 | 12 |
| 12 | TCC III 457 | 72 | 6 |
| 13 | TCC stg D 584 | 234 | 25 |

TCC = Transitional cell carcinoma of the bladder

Recur TCC = Recurrent TCC

TCC II = grade II

TCC III = grade III

TCC stg D = metastatic TCC

KS = Kidney stones

SE = Standard error

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1 Of course, the antibodies against AMF can be
2 employed to block or inhibit AMF activity thereby
3 arresting tumor invasion or metastatic proliferation
4 which depend on tumor cell motility. Availability of
5 such neutralizing antibodies also makes it possible to
6 treat such conditions as breast carcinoma and melanoma by
7 administering to a person inflicted with these
8 conditions, an effective amount of the AMF-antibodies to
9 prevent these conditions from progressing. A
10 pharmaceutical composition for treating cancer and
11 metastases is prepared by simply including an effective
12 amount of neutralizing antibodies against AMF to inhibit
13 motility of tumor cells and a pharmaceutically acceptable
14 carrier such as physiological saline, non-toxic buffers
15 and the like.

16 Means for detecting tumor aggressiveness and/or
17 metastatic activity is now also made possible by a kit
18 comprising separate containers containing (a) antibodies
19 having specific binding affinity for AMF; (b) labelled
20 AMF; (c) unlabelled AMF and instructional material for
21 performing tests utilizing the antibodies and the AMF
22 provided in the kit for determining AMF and/or receptor
23 activity in a body sample. Such accessories as
24 microtiter plates, micropipettes, means for reading
25 antibody titer and the like are routinely found in such
26 kits and may be included for convenience in the kits of
27 the present invention.

28 In summary, the present invention provides a new
29 tool for understanding mechanisms which control tumor
30 cell invasion and opens new strategies for cancer
31 diagnosis and therapy. Epithelial cells do not normally
32 exhibit invasive behavior. The motility factor described
33 herein does not affect the migration of normal blood
34 leukocytes. Therefore, a therapeutic agent aimed at

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1 inhibiting the factor described in the invention should
2 have low toxicity against normal resting tissues.
3 Pharmacologic preparations obtained in accordance with
4 the present invention which inhibit invasion of tumor
5 cells and prevent the transition from in situ to invasive
6 carcinoma could be potent cancer arresting agents.
7 Inhibitors of tumor invasion can also prevent the growth
8 of established metastases because a metastasis may need
9 to invade locally as it grows. Furthermore, such agents
10 may inhibit tumor angiogenesis. Antibodies to motility
11 factors or their receptors could be applied through
12 tissue immunohistology, radioscintigraphy, or serum
13 immunoassays to localize metastases and predict cancer
14 aggressiveness in individual patients. As gene
15 products, autocrine motility factors or their receptors
16 define a new class of oncogenes. The level of expression
17 of these genes in a patient's tumor may provide important
18 diagnostic information through monitoring the level of
19 AMF in the body sample.

20 Of course, invasion and metastases are among the
21 major causes of cancer treatment failure. The present
22 invention provides new clinical strategies to (a) detect
23 pre-invasive lesions and prevent their progression; (b)
24 accurately predict the aggressiveness of a patient's
25 tumor, and (c) identify and eradicate micrometastases.
26 One of the least understood aspects of tumor invasion is
27 tumor cell locomotion. The present invention allows the
28 determination of the role of the tumor cell motility
29 factor.

30 It is understood that the examples and embodiments
31 described herein are for illustrative purposes only and
32 that various modifications or changes in light thereof
33 will be suggested to persons skilled in the art and are
34 to be included within the spirit and purview of this
35 application and scope of the appended claims.

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1 WHAT IS CLAIMED IS:

2 1. An isolated and substantially pure mammalian
3 cell polypeptide which stimulates random locomotion of
4 producer cell, having a molecular weight >30,000 and
5 being inhibited by pertussis toxin.

6 2. The polypeptide of claim 1 having at NH₂
7 terminus, an amino acid sequence, at least in part, as
8 follows:

9 D K E L R F R D C T K S L A E A N K K.

10 3. Antibodies having specific binding affinity for
11 the polypeptide of claim 1.

12 4. A method for arresting metastatic proliferation
13 comprising administering to a host suspected of or
14 inflicted with malignant tumors an effective amount of
15 antibodies of claim 3 to inhibit tumor proliferation.

16 5. The method of claim 4 wherein said malignant
17 tumors are melanoma, breast and bladder carcinoma.

18 6. A kit for detecting tumorigenic or metastatic
19 activity in a body, comprising a container containing
20 antibodies having specific binding affinity for autocrine
21 motility factor (AMF).

22 7. A kit for determining the level of AMF cell
23 receptors comprising containers separately containing (a)
24 labelled AMF; (b) unlabelled AMF; and (c) instructions
25 for performing tests with a body sample to determine the
26 level of AMF-receptor activity.

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1 8. A method for detecting the presence of carcinoma
2 in humans comprising reacting human body sample from a
3 patient suspected of having carcinoma with a cell line
4 susceptible to AMF and determining motility induced in
5 the susceptible cell line by said body fluid.

6 9. The method of claim 8 wherein the motility
7 incuded by said human body sample is inhibited by
8 anti-AMF antibody.

9 10. The method of claim 8 wherein said carcinoma is
10 human bladder, breast or lung carcinoma.

1 / 2

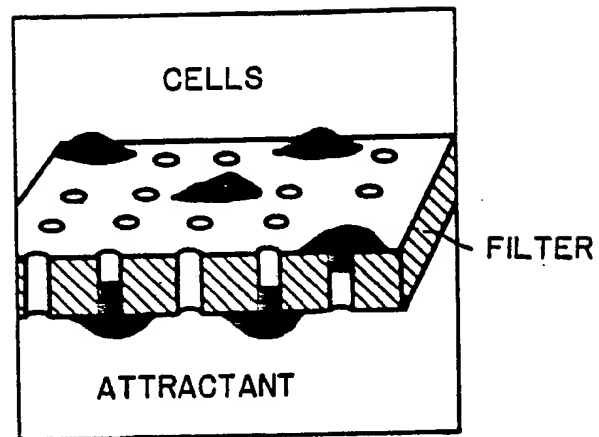


FIG. 1

2 / 2

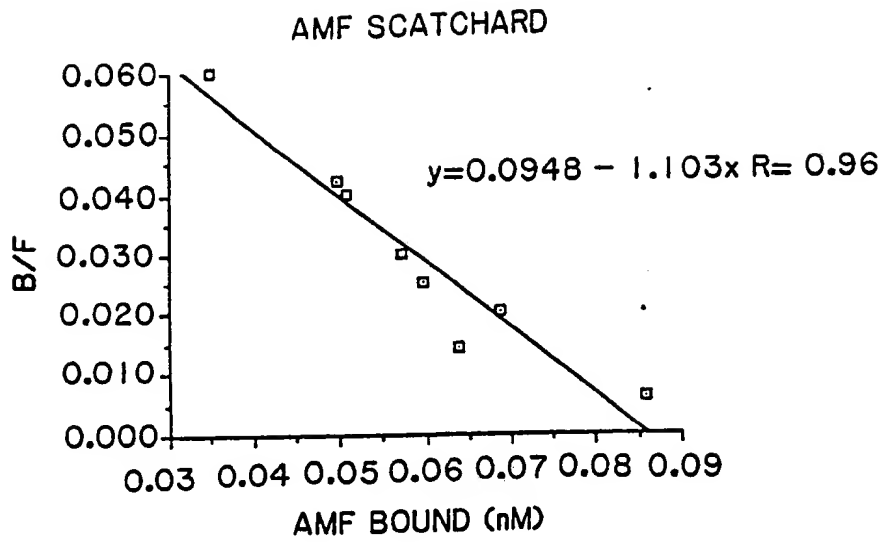


FIG. 2(a)

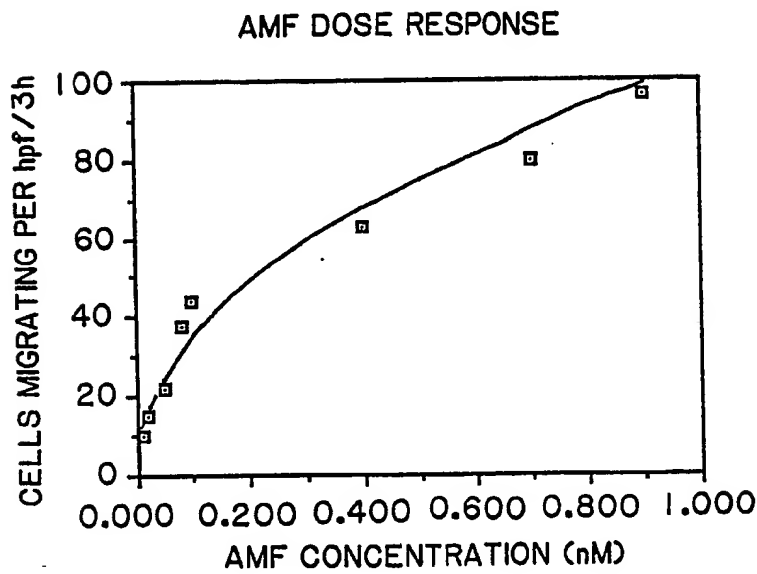


FIG. 2(b)

94 —

67 —

54kDa

43 —

18 —

FRONT →

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/01805

| | | |
|---|--|---|
| I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ | | |
| According to International Patent Classification (IPC) or to both National Classification and IPC | | |
| U.S. CL. 530/324,350,387;514/21;436/547,503;435/29 | | |
| INT. CL. (4): CO7K 15/00,7/10;A61K 37/00 | | |
| II. FIELDS SEARCHED | | |
| Minimum Documentation Searched ⁷ | | |
| Classification System | Classification Symbols | |
| US | 530/324,350,387;514/21;436/547,503;435/29 | |
| Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ⁸ | | |
| III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹ | | |
| Category [*] | Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹² | Relevant to Claim No. ¹³ |
| X | Chemical Abstract, Vol. 105, Issued July 1986 (Columbus, OH.) abstract no. 22367p (Liotta) "Tumor Autocrine motility factor" | 1-10 |
| A, P | Chemical Abstract, Vol. 108, Issued January 1988, (Columbus, OH.) abstract no. 19880g (Guirguis) "Cytokine-induced pseudopodial protrusion is couple to tumor cell migration" | 1-10 |
| A, P | Chemical Abstract, Vol. 108, Issued February 1988, (Columbus, OH.) abstract no. 53562q. "Biochemical mechanisms of tumor invasion and metastasis" (Liotta) | 1-10 |
| <p>[*] Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> | | |
| IV. CERTIFICATION | | |
| Date of the Actual Completion of the International Search | | Date of Mailing of this International Search Report |
| 29 JULY 1988 | | 01 SEP 1988 |
| International Searching Authority | | Signature of Authorized Officer |
| ISA/US | | DELBERT R. PHILLIPS |

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

| Category * | Citation of Document, ¹⁴ with indication, where appropriate, of the relevant passages ¹⁵ | Relevant to Claim No ¹⁶ |
|------------|--|------------------------------------|
|------------|--|------------------------------------|

- | | | |
|-----|---|------|
| X,P | Biochemical and Biophysical Research Communications, Vol. 146, No.1, Issued July 1987, pages 339-345, (Stracke) "Pertussis Toxin-Inhibits Stimulated Motility Independently of the Adenylate Cyclase Pathway In Human Melanoma Cells" (Bethesda, MD.) See summary. | 1-10 |
| X | Proceeding National Academy of Science, Vol. 83, pages 3302-3306, Issued May 1986, (Liotta) "Tumor cell autocrine motility Factor" See abstract .(Washington, D.C.) | 1-10 |

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